

# Extracellular mycosynthesis of gold nanoparticles using *Fusarium solani*

K. Gopinath · A. Arumugam

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**Abstract** The development of eco-friendly methods for the synthesis of nanomaterial shape and size is an important area of research in the field of nanotechnology. The present investigation deals with the extracellular rapid biosynthesis of gold nanoparticles using *Fusarium solani* culture filtrate. The UV–vis spectra of the fungal culture filtrate medium containing gold ion showed peak at 527 nm corresponding to the plasmon absorbance of gold nanoparticles. FTIR spectra provide an evidence for the presence of heterocyclic compound in the culture filtrate, which increases the stability of the synthesized gold nanoparticles. The X-ray analysis respects the Bragg's law and confirmed the crystalline nature of the gold nanoparticles. AFM analysis showed the results of particle sizes (41 nm). Transmission electron microscopy (TEM) showed that the gold nanoparticles are spherical in shape with the size range from 20 to 50 nm. The use of *F. solani* will offer several advantages since it is considered as a non-human pathogenic organism. The fungus *F. solani* has a fast growth rate, rapid capacity of metallic ions reduction, NPs stabilization and facile and economical biomass handling. Extracellular biosynthesis of gold nanoparticles could be highly advantageous from the point of view of synthesis in large quantities, time consumption, eco-friendly, non-toxic and easy downstream processing.

**Keywords** *Fusarium solani* · Extracellular biosynthesis · Gold nanoparticles · Eco-friendly · Bio-reduction

## Introduction

Nanostructured materials have been attracting considerable attention because of their unique physical and chemical properties, its potential applications in many fields such as nanocomputers (Tseng and Ellenbogen 2001), catalysis (Kim et al. 2003), optical devices (Kamat 2002), cell labeling (Wu et al. 2003), cell tracking (Parak et al. 2002), in vivo imaging (Dubertret et al. 2002), DNA detection (Taylor et al. 2000), and antimicrobial activity (Krishnaraj et al. 2012). The interaction between microorganism (e.g., bacteria, yeast, fungi) and metals have been reported as bio-reducing agent of the gold nanoparticles synthesis (Gericke and Pinches 2006). An extremophilic actinomycete *Thermomonospora* sp. has been reported to synthesize extracellular monodispersed, spherical gold nanoparticles of average size of 8 nm (Ahmad et al. 2003a). Some of the common algal genus like, *Anabaena*, *Calothrix*, and *Leptolyngbya* cyanobacteria have also been found to produce intracellular Au, Ag, Pd, and Pt nanoparticles (Brayner et al. 2007). Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus *Neurospora crassa* (Castro-Longoria et al. 2011) and bimetallic Au-core, Ag-shell nanoparticles have been synthesized using *Azadirachta indica* and *Aloe vera* leaf extract (Shankar et al. 2004; Chandra et al. 2006).

Researchers in National Chemical Laboratory (NCL) Pune, India, have investigated that the eukaryotic organisms such as fungi can be used to prepare nanoparticles and also 200 genus of fungi were investigated for this purpose. From this extensive screening study, two genera of fungi, *Verticillium* sp. (Mukherjee et al. 2001a, b) and *Fusarium oxysporum*, were found to produce nanoparticles. The latter displayed the ability to

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synthesize silver, gold and platinum nanoparticles extracellularly (Mukherjee et al. 2002; Ahmad et al. 2003b; Duran et al. 2005; Syed and Ahmad 2012). *Pelargonium graveolens* plant endophytic fungus *Colletotrichum* sp. used for the synthesis of gold nanoparticles (Shankar et al. 2003). The silver nanoparticles were also synthesized extracellularly by the silver-tolerant yeast strain MKY3 (Kowshik et al. 2003), the fungi *Aspergillus fumigatus* (Bhainsa and D'Souza 2006), *Penicillium* sp. (Sadowski et al. 2008), *Cladosporium cladosporioides* (Balaji et al. 2009), *Penicillium fellutanum* (Kathiresan et al. 2009), *Alternaria alternate* (Gajbhiye et al. 2009), *Phoma glomerata* (Birla et al. 2009), *Trichoderma viride* (Fayaz et al. 2010), *Coriolus versicolor* (Sanghi and Verma 2009) and *Fusarium solani* (Ingle et al. 2009). Recently *Penicillium rugulosum* and *Hormoconis resinae* (MTCC 368) have been identified as an exciting candidates for the synthesis of gold nanoparticles extracellularly (Mishra et al. 2012; Mishra et al. 2010) and gold triangular nanoprisms using *T. asperellum* (Mukherjee et al. 2012). Although the area concerning the use of microorganism in synthetic nanomaterials is limited, it is in great progress from the past few years.

In this present study, the fungus *F. solani* was used to synthesize gold nanoparticles. The fungal culture filtrate was used to develop an extracellular process for the synthesis of gold nanoparticles. Gold nanoparticles were observed within 24 h after  $\text{HAuCl}_4$ -solution was added to the culture filtrate. To our knowledge, this is the first report for the synthesis of gold nanoparticles using *F. solani*.

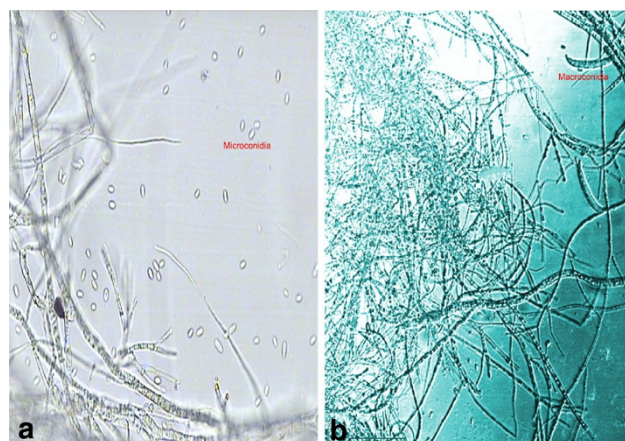
## Materials and methods

### Chemical

(Chloroauric acid) Hydrogen tetrachloroaurate (III) trihydrate,  $[\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (99.9 %)] from Alfa Aesar, was used as received. All other reagents were of analytical grade. Unless otherwise stated, double distilled water was used in all of the experiments.

### Identification of fungi

The *F. solani* cultures were collected from PG and Research Department of Plant Biology and Plant Biotechnology, Ramakrishna Mission Vivekananda College, Chennai-4. The identified fungi *F. solani* have small size of micro conidia and macro conidia (Fig. 1) characterized using Confocal laser scanning microscopy (CLSM-710, Carl Zeiss Germany).



**Fig. 1** a *Fusarium solani* microconidia; b macroconidia

### Inoculation

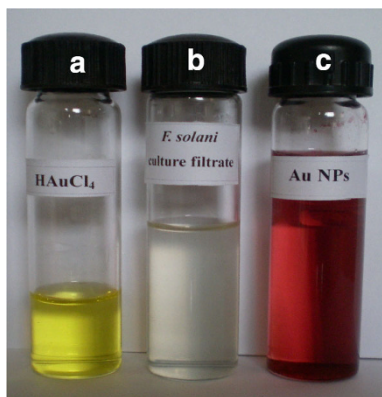
*Fusarium solani* hyphae are aseptically inoculated in the Czapek-Dox-Broth (CDB) medium containing 30 g Sucrose, 3 g Sodium nitrate, 1 g Dipotassium hydrogen phosphate, 0.5 g Magnesium sulfate and 0.01 g Ferrous sulfate, pH was adjusted to 6.5 with 0.1 N of NaOH or 0.1 N of HCl before autoclave at 121 °C and 15 lb for 20 min.

### Synthesis of gold nanoparticles

*Fusarium solani* has grown aseptically in Czapek-Dox-Broth (CDB) medium. The conical flask containing medium was incubated at 30 °C and agitated at 180 rpm for 72 h. After the incubation, the fungal filtrate was obtained by centrifugation at 7,500 rpm for 10 min. The fungal culture medium filtered through Whatman filter paper No.1 and pH of the collected filtrate was adjusted to 8.5 using 0.1 N NaOH or 0.1 N HCl. To synthesize gold nanoparticles 10 ml of  $\text{HAuCl}_4$  solution (Fig. 2a) was added drop wise to 90 ml of the culture filtrate (Fig. 2b) with a final concentration 100 ml of 250 mg/L  $\text{HAuCl}_4$  solution in an Erlenmeyer flask incubated at 30 °C and agitated at 180 rpm for 24 h. The accumulation and reduction of gold nanoparticles were observed through the turning of the color of the transparent liquid filtration to purple or pink, which indicates the formation of the gold nanoparticles (Fig. 2c).

### Characterization of gold nanoparticles

The biosynthesis of gold nanoparticles was monitored periodically by optical measurements. The liquid sample was scanned by UV–vis spectra for a wavelength range of 350–800 nm. The measurements were carried out on



**Fig. 2** a  $\text{HAuCl}_4$  solution (250 mg/L), b fungal culture filtrate, c 24-h reduction of  $\text{HAuCl}_4$  added in fungal cultured filtrate

Shimadzu spectrophotometer (Model UV-1800) operating at a resolution of 1 nm. Further, Fourier transform infrared spectroscopy (FTIR) analysis for liquid gold nanoparticles was carried out for the range of  $400\text{--}4,000\text{ cm}^{-1}$ . The Au NPs were subjected to X-ray diffraction analysis for the crystallographic structural analysis. The nano-crystallite domain size was calculated from the width of the XRD peaks using Scherrer formula  $D = 0.9\lambda/\beta\cos\theta$  (Krishnaraj et al. 2012). X-ray diffraction (XRD) analysis for a thin film sample was prepared on a glass slide ( $1\text{ cm} \times 1\text{ cm}$ ) by dropping  $100\text{ }\mu\text{L}$  of the sample on the slide, and allowed to dry for 30 min, then XRD pattern was recorded using  $\text{Cu K}\alpha$  radiation ( $\lambda = 1.54060\text{ \AA}$ ) with nickel monochromator in the range of  $2\theta$  from  $10^\circ$  to  $80^\circ$ . AFM analysis for a thin film of the sample was prepared on a glass slide ( $1\text{ cm} \times 1\text{ cm}$ ) by dropping  $100\text{ }\mu\text{L}$  of the sample on the slide, and allowed to dry for 30 min. The slides were then scanned with AFM (APE Research-model no: A100SGS). The AFM characterization was carried out in ambient temperature in non-contact mode using silicon nitride tips with varying resonance frequencies. TEM measurements were carried out to bring out the morphology of the bio-synthesized nanoparticles in terms of size and shape. Samples for TEM analysis were prepared by drop coating the nanoparticle solutions on carbon-coated copper grids at room temperature. The excess nanoparticles solution was removed with filter paper. The copper grid was finally dried at room temperature and was subjected to TEM analysis by the instrument Tecnai F20 model operated at an accelerating voltage of 200 kV.

## Results and discussion

Biosynthesis of gold nanoparticles using *F. solani* culture filtrate as reducing agent was observed around 24 h. The color of the solution was changed from

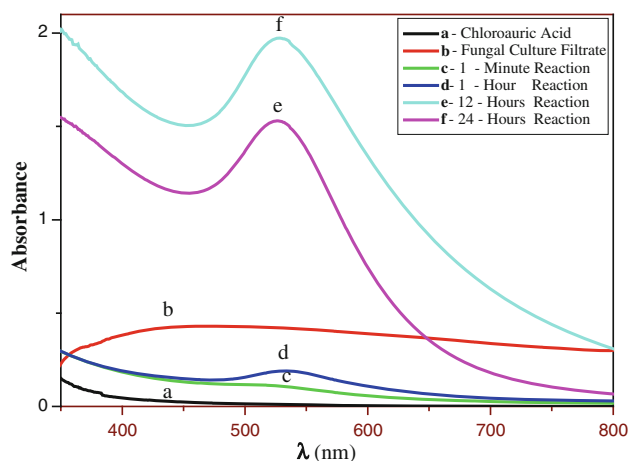
yellow to dark red which indicates the formation of the Au NPs.

### UV–vis spectroscopy

UV–vis absorption spectroscopy was used to measure the absorbance of gold nanoparticles in different intervals such as 1 min, 1, 12, and 24 h. The UV–vis spectra illustrated that there is no evidence of an absorption peak in the region of  $350\text{--}800\text{ nm}$  for the culture filtrate from *F. solani* and  $\text{HAuCl}_4$  solution. After the  $\text{HAuCl}_4$  addition with fungus filtration, a well-defined absorption peak at  $527\text{ nm}$  appears in Fig. 3 that corresponds to the wavelength of the surface plasmon resonance of gold nanoparticles (Mukherjee et al. 2002; Mulvaney 1996). Various reports have established that the resonance peak of gold nanoparticles appears around this region (Link and El-Sayed 2000; Nair and Pradeep 2002; Inbakandan et al. 2010). The sharp absorption peak at  $527\text{ nm}$  indicates the presence of gold nanoparticles in the solution.

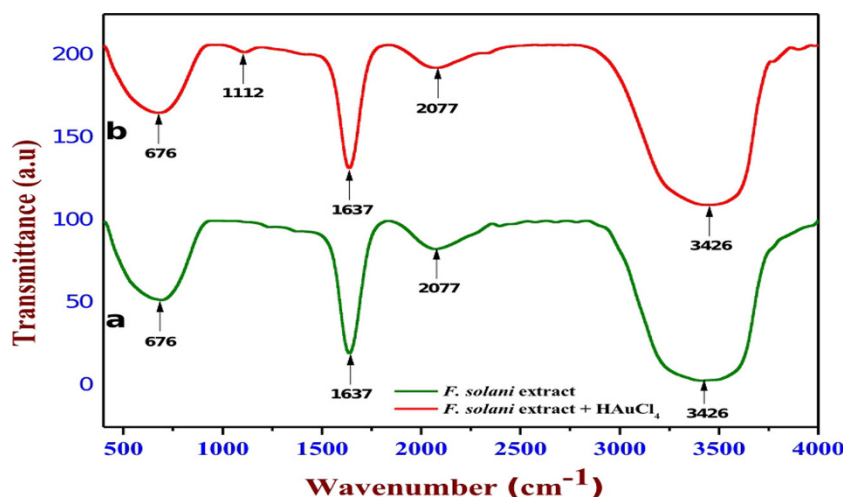
### Fourier transform infrared spectroscopy

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the  $\text{Au}^+$  ions and capping of the bio-reduced gold nanoparticles synthesized by fungal filtrate. Representative spectra of obtained nanoparticles manifests absorption peaks located at about  $3,426$ ;  $2,077$ ;  $1,637$ ;  $1,112$  and  $676\text{ cm}^{-1}$  in the region  $400\text{--}4,000\text{ cm}^{-1}$ . The FTIR spectra revealed the presence of different functional groups such as free  $\text{NH}_2$ -stretching, C–N stretching, NH-bending, C–O stretching and Cl-stretching. Curve of biosynthesized gold nanoparticles using *F. solani* extract resulted a strong



**Fig. 3** UV–visible spectrum of nanoparticles at the different timing reaction is indicated next to the respective curves with the extract of *F. solani* and aqueous solution of 250 mg/L  $\text{HAuCl}_4$  ( $527\text{ nm}$ )

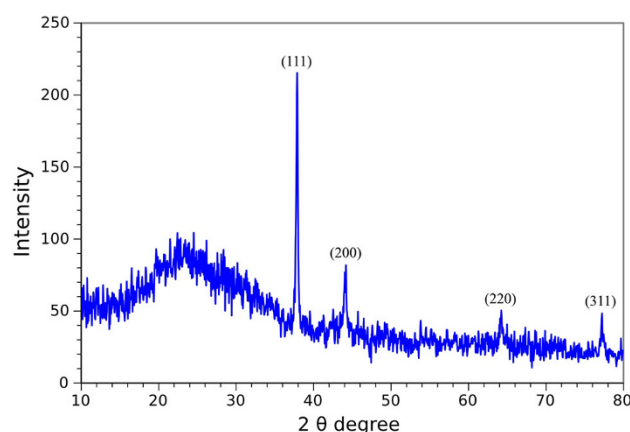
**Fig. 4** FTIR-Spectra recorded from: *a* *F. solani* cultural filtrate; *b*  $\text{HAuCl}_4$  solution (250 mg/L) + *F. solani* cultural filtrate using synthesis of gold nanoparticles



band at  $3,426\text{ cm}^{-1}$  corresponding to N–H stretching vibration of primary amines, the band at  $2,077\text{ cm}^{-1}$  corresponding to C–N stretching of any  $\text{R–N=C=S}$  (Inbakandan et al. 2010), the medium band at  $1,638\text{ cm}^{-1}$  corresponding to similar conjugation effects to N–H bending frequency, the low band  $676\text{ cm}^{-1}$  corresponding to Cl stretching. The heterocyclic ring with nitrogen-derived compounds like alkaloids and protein are present in the fungal extract and are the capping ligands for the synthesized gold nanoparticles. FTIR spectra of the culture filtrate of *F. solani* (Fig. 4a) solution show peaks at  $3,426$ ;  $2,077$ ;  $1,637$  and  $676\text{ cm}^{-1}$ . The addition of 250 mg/L chloroauric acid in fungal culture for after 24 h to took out the FTIR result. The new strong band exhibited at  $1,112\text{ cm}^{-1}$  assigned to C–O stretching. This peak may be raised due to the reduction of  $\text{HAuCl}_4$  to Au nanoparticles (Fig. 4b).

#### X-ray diffraction pattern

The XRD patterns obtained for biogenic gold nanoparticles using *F. solani* extract are shown in Fig. 5. XRD analysis showed three distinct diffraction peaks at  $38.26^\circ$ ,  $44.48^\circ$  and  $66.29^\circ$ , which can be indexed with the planes (111), (200) and (220) for the cubic face centered gold followed by a small peak at  $77.35^\circ$  indexed with (311) plane. A number of Bragg reflections corresponding to the lattice planes are observed which may be indexed based on the face-center-cubic (fcc) gold and the values are matched with standard database values (JCPDS No. 04-0784). The XRD pattern thus clearly shows that the gold nanoparticles formed by the reduction of  $\text{Au}^+$  ions by the extract of fungal culture filtrate are crystalline in nature. The similar result was found in XRD patten for intra and extra cellular biosynthesis of gold nanoparticles using *Penicillium* sp (Liangwei et al. 2011).



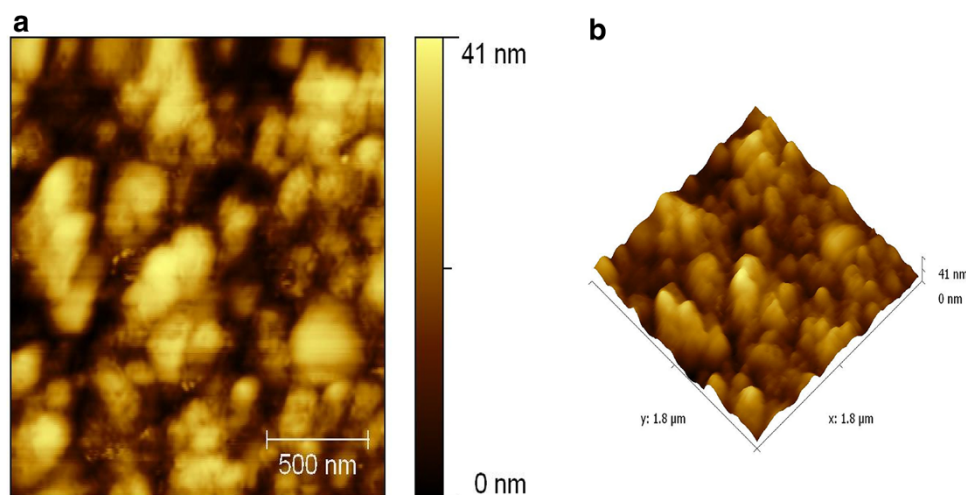
**Fig. 5** XRD-Pattern of gold nanoparticles synthesize by treating the extract of *F. solani* with  $\text{HAuCl}_4$  aqueous solution

#### Atomic force microscopy and transmission electron micrographs

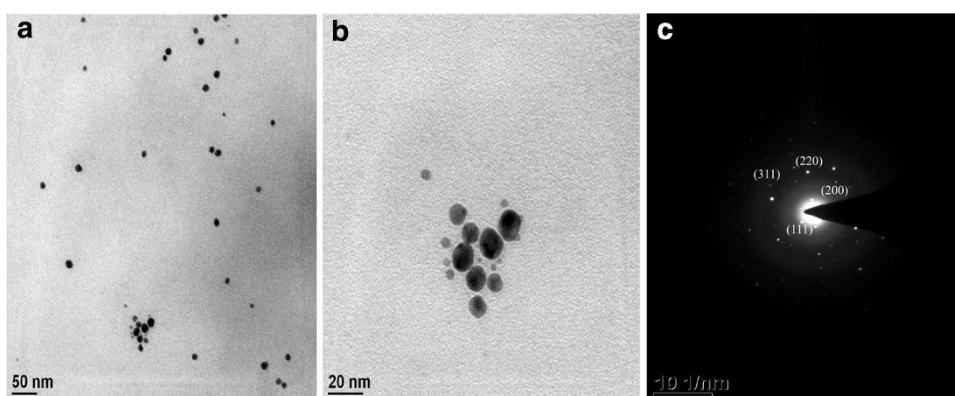
Surface morphology of the formulated gold nanoparticles was studied by AFM as shown in 2D image (Fig. 6a). The AFM 3D image (Fig. 6b) clearly indicated that, the formulated Au nanoparticles possess uniform spherical shape with the size of 41 nm. The result obtained from the TEM—transmission electron microscopy study showed a very clear indication regarding the shape and size of the nanoparticles. The gold nanoparticles formed were predominantly monodisperse with diameter ranging from 20 to 50 nm (Fig. 7a). On careful observations of various magnifications of TEM images of gold nanoparticles, it is noted that the particles are of uniform size around 20 nm (Fig. 7b). Also gold nanoparticles have an inclination of forming thin planar structures than spherical structures. Figure 7c shows the selected area electron diffraction (SAED) pattern obtained from gold nanoparticles. The Scherrer ring pattern characteristics of face centered cubic



**Fig. 6** **a** AFM-2D-images and **b** 3D-images of Au NPs synthesized by *F. solani*



**Fig. 7** TEM images of gold nanoparticles formed by reduction of  $\text{Au}^+$  ions using the extract of *F. solani*. **a** 50 nm scale, **b** 20 nm scale, **c** TEM image of selected area diffraction pattern



(fcc) for gold is clearly observed, showing that the structures seen in TEM images are nanocrystalline in nature.

## Conclusion

The present investigation indicates the extracellular synthesis of gold nanoparticles from *F. solani* culture filtrate. Synthesized gold nanoparticles were characterized by UV–vis, FTIR, XRD, AFM, and TEM analysis. TEM results revealed that the gold nanoparticles are highly stable in the diameter range between 20 and 50 nm. This simple bio-synthesis procedure for the synthesis of gold nanoparticles has several advantages such as cost-effectiveness, bio compatibility for biomedical, pharmaceutical applications and large-scale commercial production. These fungal extracellular compounds can be extended for the synthesis of other metal and metal oxide nanoparticles.

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